Neuroscience. Ionic control of sleep and wakefulness

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Abstract: Brain electrical activity differs markedly between wakefulness and sleep. Concomitant shifts in the ion composition of brain extracellular fluids were thought to be a consequence rather than a cause of the sleep-wake–dependent changes in neuronal activity. On page 550 of this issue, Ding et al. (1) report the surprising observation that ionic changes in the extracellular fluid are a potent control of sleep-wake–dependent neuronal activity.

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IONIC CONTROL OF SLEEP AND WAKEFULNESS

Perspective on Ding *et al.*,
Changes in the composition of brain interstitial ions control the sleep-wake cycle
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Brain electrical activity markedly differs between wakefulness and sleep. The concomitant shifts in the ion composition of brain extracellular fluids were thought to be mainly a consequence rather than a cause of the sleep-wake dependent changes in neuronal activity. On page … of this issue, however, Ding *et al.* [1] report the surprising observation that ionic changes in the extracellular fluid provide a potent control mechanism of sleep-wake dependent neuronal activity *in vitro* and *in vivo*.

Although the biological functions of sleep are still elusive, sleep is essential for maintaining optimal brain functioning and virtually all other aspects of body physiology that vary in synchrony with the sleep-wake cycle. In wakefulness, cortical activation is driven by wake-promoting neuromodulators primarily produced in basal forebrain, hypothalamus and brain stem [2]. Distinct neuronal groups in these brain structures express acetylcholine, hypocretin, histamine, serotonin, noradrenalin and dopamine. These cell groups are interconnected in an excitatory network, which sends convergent projections to thalamus and cerebral cortex. Together with glutamatergic and GABA-ergic (γ-hydroxy-butyric acid) systems, this so-called ascending arousal system maintains conscious and alert wakefulness, characterized by low-voltage, fast-frequency activity in the electroencephalogram (EEG) (see the figure).

During sleep, general physiology, brain circuit connectivity, and neuronal activity are broadly and profoundly changed when compared to wakefulness. In deep non-rapid-eye-movement (NREM) sleep, also referred to as slow wave sleep, the EEG in animals and humans is characterized by large slow waves of electrical activity with frequencies of roughly 1-4 Hz. The fundamental cellular phenomenon underlying these EEG waves are synchronous oscillations between “up states” and “down states” in the membrane potential of cortical neurons [3, 4]. Sleep deprivation markedly increases number, amplitude, and slope of EEG slow waves in recovery sleep [5], reflecting their robust homeostatic regulation.

Neuronal activity is regulated by the flow of ions across the cell membrane between the intra- and extracellular space. It has long been hypothesized that the breakdown during wakefulness of energy-rich compounds increases the trans-membrane conductance of potassium (gK^+) [6]. This mechanism may play a central role in the restauration of brain energy metabolism during sleep, and the homeostatic regulation of sleep need in response to prolonged wakefulness. Increased gK^+ underlies the membrane hyperpolarization in cortical neurons that produce the EEG slow oscillation in NREM sleep [6]. Furthermore, increases and decreases in extracellular K^+ concentration ($[K^+]_e$) parallel the alternating periods of depolarization and hyperpolarization of neurons and glia cells during the natural slow oscillation and under anesthesia [7].

The idea that positively charged ions may affect the sleep-wake cycle was proposed already in the 1930-ies, and calcium contained in breast milk was hypothesized to promote sleep in infants [8]. By injecting calcium-chloride ($CaCl_2$) into the pituitary of cats, sleep for up to 3 hours could be induced [9]. In fact, several researchers at that time investigated the effects of salts on sleep, including $CaCl_2$, magnesium chloride ($MgCl_2$) and potassium chloride (KCl). Quite consistently, $CaCl_2$ and $MgCl_2$ injected into the brain near the hypothalamus appeared to promote sleep, whereas KCl rather promoted wakefulness [8]. Perhaps even more fascinating, early chemical analyses of brain tissue from dogs and rabbits revealed a slight increase in brain calcium during sleep when compared to wakefulness [10]. Although these studies used a crude definition of sleep and relied on postmortem detection of ions, the data suggest a role for cations in sleep-wake regulation.

By innovative use of converging *in vitro* and *in vivo* methodologies in mice, Ding *et al.* [1] now show the importance of cations for the regulation of sleep and wakefulness. These authors found that distinct alterations in the ion composition of extracellular fluid are sufficient to control sleep-wake dependent changes in neuronal activity. Inspired by the observation that even minor modifications of ionic concentrations in the bathing solution of electrophysiological preparations *in vitro* induce stable and reproducible changes in neuronal excitability, they first asked: Does a "wake-up cocktail" containing acetylcholine, hypocretin, histamine, serotonin, noradrenaline and dopamine

reliably alter $[K^+]_e$ in cortical slices of adult mice? Indeed, superfusion of slice preparations with micro-molar concentrations of their artificial cerebrospinal fluid (CSF) quickly increased $[K^+]_e$, even when neurons were silenced with tetrodotoxin. This neurotoxin inhibits the firing of action potentials by nerve cells, suggesting that the shift in $[K^+]_e$ is not the consequence of local changes in synaptic activity. Next, they addressed the question: Do such changes also occur *in vivo*? Their experiments revealed: Yes, they do. The researchers demonstrated that EEG/EMG-defined wakefulness was linked to increased $[K^+]_e$, whereas natural sleep and anesthesia were associated with decreased $[K^+]_e$. Again, pharmacological blockade of local synaptic transmission did not affect the behavioral-state-dependent shifts in $[K^+]_e$. In addition, the sleep-wake associated changes in $[K^+]_e$ were accompanied by inverse shifts in extracellular calcium ($[Ca^{2+}]_e$), magnesium ($[Mg^{2+}]_e$), and proton ($[H^+]_e$) ion concentrations, as well as extracellular space volume. Because extracellular ionic concentrations may change as a mere consequence of the sleep-wake cycle, the key questions to be investigated remained: Can simple alterations in the extracellular ion composition wake a sleeping animal up and put a wake animal to sleep?

To tackle these questions, Ding *et al.* [1] formulated sleep-inducing and wake-inducing artificial CSF containing extracellular ions mimicking natural sleep or awake concentrations, and they examined the effects of these artificial CSF solutions on neuronal activity and extracellular space volume. The authors implanted two cranial windows symmetrically over the mice' left and right cortices, which allowed for uni-hemispheric infusion of artificial CSF and direct comparison with the contralateral hemisphere. Remarkably, these experiments revealed that directed local and brain-wide manipulations of extracellular ions control neuronal activity and extracellular volume, and are even able to override the overarching behavioral state. These findings are consistent with the preliminary observations by Demole, Cloëtta and colleagues from almost a century ago, and elegantly demonstrate that extracellular ions importantly contribute to the state-dependent regulation of neuronal activity across sleep and wakefulness.

The present study highlights the notion that sleep is an essential cellular property and that regulated changes in extracellular ion homeostasis are sufficient to alter behavioral state from sleep to wakefulness. These findings raise the following fascinating questions: Do changes in the ionic milieu also regulate enhanced sleep drive after prolonged wakefulness and sleep satiation after extended recovery sleep? Can regional ionic shifts explain local sleep? What are the effects of individual neuromodulators, rather than a cocktail, on extracellular ionic concentrations? Are the neuromodulators or the ions the stronger force for sleep-wake transitions? Could pumps and transporters that control ion flow across cell membranes provide promising new targets to treat sleep-wake disorders? Future work may also investigate the effects of REM sleep on extracellular ions, a sleep state characterized by distinct neurophysiological features that are partly common to both, sleep and wakefulness [11].

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Caption to Figure

Wakefulness and slow wave sleep differ markedly in polysomnographic signals (EEG: electroencephalogram; EOG: electrooculagram; EMG: electromyogram), brain neuromodulator activity, extracellular ion concentrations and interstitial volume. ↑: relative increase; ↓: relative decrease (see text).

Wakefulness

Polysomnographic signals	Neuromodulator activity	Extracellular space
EEG EOG EMG 1 s	Acetylcholine ↑ Hypocretin ↑ Histamine ↑ Serotonin ↑ Noradrenaline ↑	$[K^+]_e$ ↑ $[Ca^{2+}]_e$ ↓ $[Mg^{2+}]_e$ ↓ $[H^+]_e$ ↓ Volume ↓

Slow wave sleep

Polysomnographic signals	Neuromodulator activity	Extracellular space
EEG EOG EMG 1 s	Acetylcholine ↓ Hypocretin ↓ Histamine ↓ Serotonin ↓ Noradrenaline ↓	$[K^+]_e$ ↓ $[Ca^{2+}]_e$ ↑ $[Mg^{2+}]_e$ ↑ $[H^+]_e$ ↑ Volume ↑

Figure 1